REMARKS

Entry of this Amendment and reconsideration of the rejection of the claims is respectfully requested.

New claims 47-70 have been added. The new claims are supported throughout the specification including at page 21, line 4 to page 23 line 33. Applicants submit the newly presented claims do not raise any issue of new matter.

35 U.S.C. § 103(a)

The Examiner has rejected claims 2, 4-5, 8-14, 17-20 and 24-46 under 35 U.S.C. § 103(a) as being unpatentable over Hendrickson in view of Gibson and Gold. The Examiner contends that Hendrickson teaches a method comprising forming a capture molecule - target complex, adding a nucleic acid moiety containing a detector molecule specific for the target, washing the complex to remove test materials (e.g. nucleases) and amplifying and quantifying nucleic acid by PCR. The Examiner contends Gibson discloses the use of real time PCR using sequence specific non-primer probes. The Examiner also contends that Gold teaches that aptamers have many advantages over antibodies and can readily be employed in assays in place of antibodies. The Examiner also contends that the step of washing to remove nucleases is taught by Hendrickson and that discovering a new property associated with a step is not a basis for patentability. Applicants respectfully traverse.

Applicants claims are directed to a method for detecting a target molecule in a sample that may contain the target molecule and a nuclease comprising exposing the sample to a capture antibody or target molecule binding fragment thereof whereby a capture antibody, or fragment thereof, forms a complex with the target molecule; adding to the complex from step (a) an RNA or DNA aptamer detector molecule which binds to the target to form a ternary complex; washing the complex to remove nucleases; amplifying the aptamer by PCR and quantitating or detecting the PCR amplified DNA using a detectable non-primer probe and real-time PCR; wherein quantitating or detecting PCR amplified DNA quantitates or detects the target molecule. In another embodiment, the method provides for detection of target molecules when present at a concentration of about 0.005 to about 5000 pg/mL.

In order to establish a prima facie case of obviousness, three basic criteria must be met, namely: 1) the references when combined must teach or suggest all of the claim limitations; 2) a suggestion or motivation to modify the references or combine the reference teachings; and 3) the references when combined must provide a reasonable expectation of success. Applicants submit that all of these requirements have not been met.

1. The references when combined do not teach all of the elements of the claimed invention.

Applicants submit that even when all of the references are combined, they do not disclose all of the elements of the claimed invention. Applicants have claimed a method for detecting a target in a sample containing a target and a nuclease including a step of washing the complexes formed to remove nucleases. Applicants have also claimed a method that can detect the presence of a target in a sample at a concentration of .005 to 5000 pg/mL.

The Hendrickson et al. reference does not disclose a method directed to detecting a target in a sample that may contain a target and a nuclease and including a step of washing the complexes to remove the nuclease. The Hendrickson et al. reference et al. is not concerned with the presence or absence of nucleases, because it does not contemplate, teach or suggest the use of aptamers in order to capture or detect the target molecule. Thus, the presence of nucleases and the need to remove nucleases is not addressed by Hendrickson et al. Moreover, Hendrickson suggests that washing steps can and should be eliminated in order to simplify the assay described therein further. See page 528, second column, first full paragraph.

The deficiencies of the Hendrickson et al. reference are not remedied by reference to Gibson et al. or Gold et al. Gibson et al. is also silent on the use and/or amplification of aptamers and does not discuss any concerns relating to nucleases in the sample. Gold et al. discusses the use of aptamers in assays for detecting target molecules and discloses that the conditions in which the nucleic acid antibody is employed the nucleic acid is substantially resistant to degradation. (Col. 27, lines 50-55). Gold et al. does not teach or suggest that step of washing to remove nucleases would be sufficient and should be employed to remove nucleases to protect the aptamer from degradation.

Thus, Applicants submit that even when all of the references are combined, they do not teach all of the elements of the claimed invention, in particular, washing the complexes to remove nucleases.

Applicants further submit that the references, either alone or in combination, do not teach a method for detecting a target molecule that can detect the presence of a target molecule in a sample at concentrations of .005 to 5000 pg/mL. Hendrickson et al. teach that immuno PCR is sensitive assay and can detect target molecules at 1 fg. using antibodies for both capture and detection. However, there is no teaching or suggestion that in Hendrickson et al. that the antibody used to detect the target molecule can be replaced with a aptamer and that the PCR can be replaced by detection of aptamer with a non-primer probe and real-time PCR and maintain the ability to detect target molecules at picogram levels. This is especially true when the sample is a biological sample may contain nucleases or other interfering substances.

The deficiencies of Hendrickson et al. are not remedied by the Gibson et al. and Gold et al. references. The Gibson et al. reference shows that real-time PCR can be sensitive to detecting low copy numbers of mRNA, but does not teach or suggest that the same sensitivity can be obtained when the nucleic acid to be detected is an aptamer and the aptamer is bound to an antibody target molecule complex in a sample that may contain nucleases or other interfering substances.

Gold et al. also does not teach or suggest that aptamers could be substituted in an assay for detection of a target molecule and achieve the same level of sensitivity for detection of a target molecule in a sample that may contain nucleases or other interfering substances. There are no working examples in Gold et al. showing that an aptamer can be substituted in an assay for detecting a target molecule and achieve the same level of sensitivity.

Thus, Applicants submit that the cited references either alone, or in combination, do not disclose all of the elements of the claimed invention. In the least, the cited references when combined do not teach or suggest a method for detecting a target molecule in a sample that may contain nucleases, including a step to wash the complexes to remove nucleases and do not teach or suggest a method that has the dynamic range of detection in biological samples that may contain nucleases or other interfering substances as claimed by Applicants.

2. The Examiner has not established that there is a motivation to combine the cited references or modify the Hendrickson et al. reference with the Gibson et al. and Gold et al. references.

Applicants submit that the Examiner has not established a motive to combine these references and has not properly taken into account evidence that the art taught away from the methods as claimed by Applicants. In fact, "[A] rejection cannot be predicated on the mere identification . . . of individual components of the claimed invention. Rather, particular findings must be made as to the reason the skilled artisan, with no known knowledge of the claimed invention, would have selected these components for combination in the manner claimed." Ecolochem Inc. v. Southern Calif. Edison Co., 227 F3d 1361, 1375 (Fed. Cir. 2000). "Obvious to try" is not the standard. Ecolochem at 1374. Applicants submit that cited references implicitly or explicitly do not provide motivation to combine these references.

The Hendrickson et al. reference is directed to an immuno PCR assay to detect multiple analytes utilizing antibodies in combination with a PCR detection method. The reference itself does not teach or suggest that antibodies could be substituted with aptamers or that PCR can or should be substituted with real-time PCR. The reference does discuss alternative methods of detection, but those alternative methods of detection were using direct detection of ligands or the use of enzyme labels incorporated into nucleic acids or nucleotides.

The Hendrickson et al. reference further indicates that antibody reagents labeled with fluorescent labels were not practical, because of overlapping signals from different labels and difficulties in discriminating signal intensities at various analytic concentrations that affect quantitation and sensitivity (page 527, 2nd column, 3rd paragraph) indicating that not all other detection methods are suitable or can easily be substituted into immuno PCR, especially with biological samples. In addition, Henderickson et al. discusses the simplicity of their method and minimizing the number of handling steps (p. 528 bottom of column 1 and top of column 2). Hendrickson et al. does not discuss the problem of nuclease contamination and how it might affect an assay utilizing aptamers and real-time PCR. In fact, Hendrickson suggests wash steps to remove sample materials may not be necessary and it may be desirable to eliminate such wash steps.

The Gibson et al. reference also does not provide motivation to combine the cited references. Gibson describes use of real-time PCR to detect mRNA samples for use in detecting expression in gene therapy. Gibson et al. does not teach or suggest this assay should be substituted for other PCR assays in immuno PCR methods. Gibson et al. describes an assay that is more complicated and requires more steps. Applicants submit that one of skill in the art would not be motivated to substitute this method of detection in the methods of Hendrickson et al. due to the increase in complexity.

Moreover, the Gibson et al. reference teaches using a hybridization probe that is fluorescently labeled that under certain circumstances can result in a lack of sensitivity and dynamic range due to overlapping fluorescent spectra or high background as further discussed in Hendrickson et al. In addition, Gibson employs internal control DNA that may not be readily incorporated into immuno PCR reactions, especially those involving biological samples. Finally, Gibson et al. is silent the problem of nucleases and other contaminants in biological samples and does not teach or suggest any methods for addressing the presence of nucleases or the affect of the presence of nucleases on the sensitivity and/or dynamic range of the assay.

The Gold et al. reference also not provide motivation to combine the cited references. Gold et al. describes the selection and identification of aptamers and further suggests that aptamers can be employed in the same assays as antibodies under conditions where nucleic acids are resistant to degradation. (Col. 27, lines 50-60). However, Gold et al. does not suggest substituting detection methods in immuno PCR with real-time PCR. There is no showing in Gold et al. that this substitution can be made and the same sensitivity and/or dynamic range of the assay be achieved, especially in biological samples. Thus, Applicants submit one of skill in the art would not be motivated by Gold et al. to employ aptamers in the PCR assay of Hendrickson et al. in samples containing nucleases. Moreover, Gold et al. is silent on the use of real-time PCR as a substitute for PCR in an amino PCR assay.

As discussed previously, the Williams et al. reference teaches away from the use of aptamers by indicating that use of aptamers is limited by their sensitivity to nucleases in biological samples. The Examiner contends that Gold teaches washing of complexes to produce amplifyable nucleic acids. However, the section of the patent cited to by the examiner is directed to releasing the selected aptamers from a bound complex with protein that have been bound to

nitrocellulose filters. There is no teaching or sugestion of the need for a wash step to remove nucleases or other interfering molecules but rather this step is directed to recovering the aptamer for amplification and identification of the selected aptamer that is specific for that particular protein.

In summary, Applicants submit that there is no motivation to combine all the cited references. The cited references do not provide the motivation to combine and substitute both the antibodies and PCR of Hendrickson with the aptamers of Gold et al. and real-time PCR of Gibson et al. In fact, Williams et al. teach away from substituting aptamers in situations where degradation might be present, such as due to nucleases in biological samples. In addition, Applicants submit that one of skill in the art would not be motivated to substitute the real-time PCR of Gibson et al. for that of Hendrickson et al., because it is more complicated, uses fluorescently labeled probes that can affect sensitivity and uses internal competitor DNA that may also affect sensitivity when using biological samples. Thus, Applicants respectfully request withdrawal of the rejection on this basis.

3. The references do not provide a reasonable expectation of success.

Applicants claims are directed to an assay for detecting a target molecule in a sample that may include nucleases using a detection method using an aptamer and real-time PCR. Applicants have also claimed an assay that detects a target molecule in a biological sample at a range of concentration of .005 pg/mL to 5000 pg/mL.

Applicants submit that none of the cited references teach or suggest that the antibody of Hendrickson can be substituted with an aptamer especially in samples that may contain nucleases to detect and sensitively detect or quantitate the target molecule. Both Hendrickson et al. and Gibson et al. are silent on whether aptamers could be utilized in immuno PCR assay. Gold et al. teaches that aptamers can be utilized in assays in place of antibodies, but under conditions where the nucleic acid is resistant to degradation. Williams et al. teaches away from the use of aptamers when samples contain nucleases. Moreover, biological samples could contain other materials that could inhibit or degrade aptamers. Thus, Applicants submit that one of skill in the art would not have a reasonable expectation of success that aptamers could be substituted for

antibodies in an assay for detecting target molecule in a biological sample or in a sample that may contain nucleases.

In addition, the cited references in combination do not teach or suggest that both the antibodies and the PCR assay of Hendrickson et al. could be substituted with aptamers and real time PCR and achieve the sensitivity and dynamic range of detection as claimed by Applicants. Hendrickson et al. teaches that addition of more steps, reagents and washing can decrease sensitivity and that not all detection methods provide the same sensitivity, especially those involving fluorescent labeled reagents (page 527, col. 2, 3rd paragraph; p. 528, col. 2, top of page and first paragraph). The Gibson et al. method includes multiple steps and discusses the sensitivity of the assay may also be limited by overlapping spectra of the reporter dyes (p. 996, col. 1, 2nd paragraph). Gibson et al. also employs internal competitor DNA that may interfere with the sensitivity of detection in complex biological samples. Finally, substituting aptamers for DNA labeled antibodies in biological samples or samples that may contain nucleases in the least, as taught by Williams et al., can limit the sensitivity of the assay. Thus, Applicants submit that when all of these references are considered as a whole, one of skill in the art would not have a reasonable expectation of success that substitution of both aptamers and real-time PCR in the method Hendrickson et al. would provide an assay for detecting a target molecule with a high degree of sensitivity, especially in a biological sample.

The Examiner rejected claims 2, 4, 5, 8-14, 17-20 and 46 under 35 U.S.C. § 103(a) as being unpatentable over Cubicciotti in view of Gibson et al. The Examiner contends that Cubicciotti teaches a method for detecting a target using a capture molecule, adding to the complex a nucleic acid containing a detector molecule, washing the complex and amplifying nucleic acid moiety (aptamer) by PCR and quantitating or detecting PCR amplified material. The Examiner further contends Gibson et al. teach using real-time PCR with a detectable non-primer probe. Applicants respectfully traverse.

Applicants claims are directed to a method for detecting a target molecule in a sample that may contain the target molecule and a nuclease comprising exposing the sample to a capture antibody or target molecule binding fragment thereof whereby a capture antibody, or fragment thereof, forms a complex with the target molecule; adding to the complex from step (a) an RNA or DNA aptamer detector molecule which binds to the target to form a ternary complex; washing

the complex to remove nucleases; amplifying the aptamer by PCR and quantitating or detecting the PCR amplified DNA using a detectable non-primer probe and real-time PCR; wherein quantitating or detecting PCR amplified DNA quantitates or detects the target molecule. In another embodiment, the method provides for detection of target molecules when present at a concentration of about 0.005 to about 5000 pg/mL.

In order to establish a prima facie case of obviousness, three basic criteria must be met, namely: 1) the references when combined must teach or suggest all of the claim limitations; 2) a suggestion or motivation to modify the references or combine the reference teachings; and 3) a reasonable expectation of success. Applicants submit that all of these requirements have not been met, in the least because, the cited references when combined do not disclose all of the elements of the claimed invention.

Applicants submit that the Cubiciotti reference does not disclose an assay for detecting a target in a sample that may contain nucleases employing a capture antibody and an aptamer detector molecule, washing to remove nucleases, quantitating or detecting the aptamer using real-time PCR quantitating or detecting the target molecule. The section of the patent cited by the Examiner is in an Example entitled "Selection of a Synthetic Defined Sequence Segment for the Ability to Stabilize a Peptide Drug". (Col. 227, lines 63-65). The patent further indicates the example describes selecting a defined sequence segment that binds a therapeutic peptide and attenuates peptide degradation (col. 228, lines 20-30). The method described at column 229, lines 5-52 is a method for selecting an aptamer that decreases the degradation of chimeric F(ab) fragment anti-7E3 (col. 229, lines 15-20). The F(ab) fragment anti-7E3 is the therapeutic protein used to inhibit platelet aggregation. In contrast to the assertion of the examiner, the therapeutic protein is not peptide 7E3 but rather is the antibody fragment itself. The method of this example involves selecting aptamers that bind to to the protein and prevent degradation of the protein. The selected aptamers were isolated by affinity chromatography and were amplified and sequenced (col. 229, lines 34-60). This process does not disclose a capture antibody for binding to a target molecule, a detectable aptamer that binds target molecule in a target molecule:capture antibody complex, washing to remove nucleases, amplifying the aptamer, quantitating or detecting the PCR amplified DNA using a detectable non-primer probe and quantitating or detecting the PCR amplified DNA to quantitate or detect the target molecule. Moreover, since

this method is not a method to detect a target molecule and because there are no actual examples, there is no discussion of detecting or quantitating the target molecule in a range of .005 pg/mL to 5000 pg/mL.

The deficiencies of the Cubiciotti et al. references are not remedied by reference to Gibson et al. Gibson et al. does not disclose using a capture antibody or an aptamer detector molecule that binds to the target molecule or washing the complexes to remove nucleases. Gibson discusses detection of a mRNA molecule, but does not teach or suggest that the level of detection could be maintained in other assays. Thus, Applicants submit the cited references when combined do not teach or suggest all of the elements of the claimed invention. Applicants, therefore, request the withdrawal of the rejection of the claims on this basis.

The Examiner also rejected claims 24-45 as being unpatentable over Cubiciotti et al. in view Gibson and further in view of Hendrickson et al. The Examiner contends that Cubiciotti teaches the method of claim 46, but is silent regarding the concentration of the target molecule. The Examiner contends that it would be obvious to apply the well known detection sensitivity of Hendrickson to detect molecules at a concentration equal to or less than 1 pg/mL. Applicants respectfully traverse.

Applicants submit the Cubiciotti et al. reference does not teach the method of claim 46. As discussed previously, the cited portions of the Cubiciotti et al. reference are directed to a method of selecting an aptamer that binds to a protein and prevents degradation of this protein. The protein is F(ab) fragment of anti-7E3. This protein is the protein to be protected and does not serve as the capture antibody. Moreover, the aptamer is amplified by PCR not to quantitate or detect the protein, but to obtain aptamer for use in conjunction with the antibody to prolong half-life of antibody fragment in vivo.

The deficiencies of the Cubiciotti et al. references are not remedied by reference to Hendrickson et al. or Gibson et al. There is no teaching or suggestion in either reference that the antibodies and PCR of Hendrickson et al. could be substituted with aptamers and real-time PCR and achieve the same level of sensitivity, especially in samples that may contain nucleases.

Thus, Applicants respectfully request withdrawal of the rejection on this basis.

SUMMARY

Applicants submit that the claims are in condition for allowance and notification to that effect is earnestly solicited. The Examiner is invited to contact Applicants' representative if prosecution may be assisted thereby.

Respectfully submitted,

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